Amendments to the Specification

Referring to the published application no. 2002-0198372 for all amendments to the specification following.

Please replace paragraph [0001] with the following amended paragraph:

[0001] This application is a continuation-in-part of U. S. Ser. No. 08/691,090, now issued US Patent No. 6,011, 148, which is incorporated herein by reference.

Please replace paragraph [0010] with the following amended paragraph:

[0010] The present invention provides automatable methods for purifying plasmid DNA from cells. The methods involve use of a static mixer to mix the cells with a lysis solution to provide controlled, gentle mixing of the cells with the lysis solution. Static mixers are further used to mix the resulting lysis mixture with a precipitation solution to precipitate out cell debris and other contaminants, including chromosomal DNA. This is typically followed by an additional step of centrifugation to remove the precipitated material. The methods of the invention are sufficient to provide a purified DNA solution that does not require complex purification steps (e.g., ultrafiltration) prior to application to an ion exchange chromatography to produce a final product.

Please replace paragraph [0029] with the following amended paragraph:

[0029] FIG. 1A is a schematic diagram of a system of the present invention. The illustrated system can be conveniently used for isolation of plasmid DNA from bacterial cells. The various solutions are flowed through the mixers and lines of the system using pumps (e.g., peristaltic pumps) or pressure according to standard techniques. A tank 10 containing cells is linked by line 20 to static mixer 30. The cells (typically *E. coli*) are usually first pelleted from a culture according to well known techniques. After pelleting, the cells to a ~~denisty~~ density of up to 75% solids, they can be frozen for future plasmid preparation or used directly in the methods of the invention. Typically, the cells are resuspended in a suitable solution (e.g., 25 mM Tris-HCl, pH 8, 10 mM EDTA, 50 mM dextrose) at room temperature with stirring at 150 rpm for 1 h. Typically, the cells are resuspended in about 5 liters per kilogram (kg) of cell paste. Alternatively, static mixers can be used for this purpose. RNase can also be added at this time (e.g., 24,000 Kunitz units RNase/kg cell paste) to decrease the amount of RNA in later steps.

Please replace paragraph [0032] with the following amended paragraph:

[0032] A linear velocity of 0.38 to 2.3 feet per second gives acceptable product quality when using a 2" diameter, ~~24~~ 24 element, laminar flow static mixer from Kenics with an overall length of about 6 feet (corresponding to a Reynolds number from 50 to 500).This linear velocity range permits sufficient mixing to thoroughly lyse the cells and yet not be so high that genomic DNA is sheared to a size that is problematic in later purification steps. At a 0.7 feet per second linear velocity the flow rate in a 2" diameter mixer is typically 22 liters per minute.

Please replace paragraph [0033] with the following amended paragraph:

[0033] The lysis mixture exiting the static mixer **30** then flows through line **60** to static mixer **70**. A tank **80** containing a precipitating solution is connected through line **90** to line **60**. The precipitating solution is used to precipitate proteins, chromosomal DNA and cell debris. Typically, the solution will contain potassium acetate. A suitable precipitating solution is 3M potassium acetate, adjusted to pH 5.5, with acetic acid (.about.5M acetate final). As with the first static mixer, the intersection between lines **60** and **90** can be adjusted so that the lysis mixture and the precipitation solution enter the static mixer [[**30**]] **70** essentially simultaneously. Similar linear velocities are used to ensure sufficient mixing to thoroughly precipitate the proteins and cellular debris and yet not be so high that genomic DNA is sheared to a size that is problematic in later purification steps. Typically, approximately 5 liters of precipitating solution is used per kg of cell paste.

Please replace paragraph [0034] with the following amended paragraph:

[0034] After exiting static mixer [[**30**]] **70**, the precipitating solution flows through line 100 to centrifuge 110. In some embodiments, a tank 120 containing a buffer solution is connected to line 100 through line 130. The buffer solution is used to raise the pH of the solution to minimize acid catalyzed de-purination of the DNA and to condition the material for binding onto the anion exchange column, e.g., a pH is the range of 6 to 9, preferably from 7 to 8.5. A useful buffer solution for this purpose is 1 M Tris. More concentrated solutions can be used to adjust the pH, however the use of a more diluted buffer solution has other benefits. A diluted buffer reduces the viscosity of the solution going into the centrifuge to yield better clarity. It also decreases the ionic strength of the solution, such that it can be loaded directly onto the anion exchange column.

Please replace paragraph [0040] with the following amended paragraph:

[0040] A chromatography column is packed with an anion exchange chromatography resin. The optimal capacity of the column is determined empirically based on the resin used and the size of the nucleic acid to be purified. The column is packed under low pressure, typically less than about [[7]] 0.7 bar. The pressure will depend on the resin used, and will usually be according to the manufacturer's specifications. Normal column operating pressure may be lower where the resin pore size is smaller, to limit trapping of the nucleic acid in the resin pores. Thus, for resins without pores, column operating pressure may be increased. The column is packed at about twice the anticipated flow rate in accordance with conventional techniques.

Please replace paragraph [0041] with the following amended paragraph:

[0041] The nucleic acid sample is loaded onto the column in a loading buffer comprising a salt concentration below the concentration at which the nucleic acid would elute from the column. Typically, the salt concentration will be about 10 to 50 mS/cm, depending on the resin used. For weaker anion-exchange resins, a lower conductivity solution will be used, whereas for stronger anion-exchange resins, a higher conductivity solution will be used. The column will then be washed with several column volumes of buffer to remove those substances that bind weakly to the resin. The nucleic acid is then eluted from the column using either one or more step increases in the saline concentration or a shallow continuous saline gradient according to conventional methods, e.g., using up to 1.5M NaCl in a Tris (pH 8.5) buffer. Collection of the nucleic acid from the step elution method is accomplished by directly pooling the plasmid into a vessel, based on absorbance, conductivity, volume or time. For eluant from the continuous gradient method, similar monitoring can be used or sample fractions can be collected and analyzed. For intermediate scale preparations (e.g., from about 100 mg to about 3 grams nucleic acid), fractions will typically be at least 50 ml to 2 liters where the nucleic acid peak is expected, then increased in volume past the expected peak. Analytical determinations of nucleic acid yield and purity are performed on each fraction. In addition, Limulus ameobocyte lysate (LAL) analyses may be performed on each fraction to determine residual endotoxin levels in each fraction. Fractions containing high levels of nucleic acid and low endotoxin are pooled. For large scale preparation, step elution is preferred. For example, when using a resin as described in the Examples herein, the plasmid DNA loading solution conductivity is about 50 mS/cm, and the plasmid DNA is eluted at about 59 mS/cm. The resulting nucleic acid sample may again be filtered through a [[0.21]] 0.2 μm filter to control the number of microbial organisms.

Please replace paragraph [0042] with the following amended paragraph:

[0042] The preferred method for purifying plasmid DNA at larger scale is to load the lysate directly onto the ion exchange chromatography column after clarification and neutralization. This can be done to avoid additional purification steps, and greatly simplifies the process. In this "direct load" process, after the lysate is clarified by centrifugation, further debris may be removed by, e.g., decanting through a depth filter. The pH and conductivity is then adjusted to the appropriate values, which depend on the anion exchange resin used. A preferred resin is TMAE Fractogel 650M resin (EM Separations Technology, Wakefield, R.I., US Associate of E. Merck, Darmstadt, Germany). TMAE Fractogel 650M is a tentacle ion exchanger having trimethylaminoethyl functional groups (TMAE) covalently attached to hydroxyl groups of a synthetic methacrylate based polymeric resin backbone. With this resin, [[he]] the pH is adjusted to about 8.5 and the conductivity is adjusted to less than about 50 mS/cm. This can be accomplished by adding about 0.6 volumes of 1M Tris per volume of lysate or by diluting 0.25-0.3 fold with water, then adding Tris base powder to a final concentration of 0.62M.

Please replace paragraph [0044] with the following amended paragraph:

[0044] In some embodiments, ultrafiltration may be used before the step of ion exchange chromatography. Typically, ultrafiltration is carried out as described in WO 98/05673, which corresponds to U.S. Ser. No. 08/691,090, now US Patent No. 6,011,148, supra.

Please replace paragraph [0063] with the following amended paragraph:

[0063] An inoculum of *E. coli* containing plasmid p4119 (FIG. 3) was prepared from frozen stock by the addition of 1 ml of frozen (-80°C.) bacterial culture to a 500 ml foam-plugged flasks containing 100 ml TB broth (Sambrook et al., 1989) supplemented with carbenicillin (100 μg/ml). Cultures were incubated at 37°C. and shaken at 220 rpm for approximately 6 hours. Culture growth was determined by visual inspection or by determining OD_{600}, whereby OD values between 0.5 and 5 were deemed acceptable. 5 ml of this culture was used to inoculate each of 4 bioreactors containing 10L TB media supplemented with carbenicillin ~~(100 lg/ml)~~ (100 μg/ml) and with 1 ml/10L Mazu DF204 antifoaming agent. These cultures were incubated at 37° C. and stirred initially at about 300 rpm. The cultures were aerated and dissolved oxygen was controlled via cascade control loops, agitation, airflow, and oxygen enrichment to an average of about 40% saturation. Cultures were incubated for about 10 to 16 h. After incubation, cell content of each culture was determined by OD_{600}; OD_{600} values ranged from 16 to 18. Cells were harvested by centrifugation in a refrigerated Carr continuous flow centrifuge.

Please replace paragraph [0064] with the following amended paragraph:

[0064] The cell pellets were spread into thin sheets and frozen at -80° C. until used for further plasmid purification. 3.2 Kg of the cell pellet was resuspended in 16L Solution I (25 mM Tris-HCl, pH 8, 10 mM EDTA, 50 mM dextrose) at room temperature with stirring at 150 rpm for 1 h. RNase digestion was achieved by the addition of RNase (305 mg RNase/Kg cell paste) and incubating the solution on ice for 2 hrs. Cells were lysed by the addition of the cells to 32L Solution II (0.2N NaOH/1%SDS) in an ice bath. The solution is stirred using a Bow-Tie Stirrer (Cole Parmer, Vernon Hills, Ill.) for 25 min. This solution was then neutralized and cell debris and chromosomal DNA were precipitated by the addition of 16L ice-cold Solution III (3M potassium, [[SM]] 5M acetate, pH 5.5). The solution was mixed with a Bow-Tie Stirrer on ice for 25 min.

Please replace paragraph [0065] with the following amended paragraph:

[0065] The precipitated material was removed from the neutralized cell lysis solution by centrifugation. The solution was aliquoted into [[IL]] 1L centrifuge bottles and centrifuged at 5300 rpm for 20 min at 2° C. The supernatants were then decanted through two layers Miracloth (CalBiochem, La Jolla, Calif.) arranged at 90° to each other, into a container at room temperature. The decanted supernatants were then filtered through 1.2 and [[0.21]] 0.2 µm filters arranged in series. As an alternative to centrifugation at this stage, precipitated material may be removed by filtration through a diatomaceous earth material such as Celite® HYFLO SUPER CEL® (Celite Corp., Lompoc, Calif.). See U.S. Pat. No. 5,576,196.

Please replace paragraph [0069] with the following amended paragraph:

[0069] Spectrophotometric analysis was performed at wavelengths of 250, 260, and 280 [[m]] nm. Typical ratios for purified DNA are OD_{260}/OD.sub.250>1.1, and OD_{260}/OD_{280}>1.9. A total of 2.307 g of plasmid DNA was isolated and purified in the above procedure, having OD_{260}/OD_{250} of 1.1047 and OD_{260}/OD_{280} of 1.9290.

Please replace paragraph [0083] with the following amended paragraph:

[0083] Approximately 40 L of fermentation broth yields about 2.2 kg of cell paste. After re-suspension of the cell paste, lysis and precipitation, approximately 40 liters of solution were ready for clarification by centrifugation. Centrifuging in a non-continuous centrifuge (Sorvall RC3b) at 7500 x g for 25 minutes removed the solids and yielded a clarified product. Tris base (solid) was added to adjust the pH of the clarified product to 8.5 (a final concentration of 0.67 M). After Tris base addition, the conductivity decreased from 53 mS/cm to 50 mS/cm. The neutralized lysate was filtered in series with a nominal 0.2 µm glass filter (Sartorpure GF) and an absolute [[0.2 m]] 0.2µ nylon filter (Pall Ultipor N_{66}) (5 ft^2 each) to reduce bacterial load and endotoxin levels.

Please replace paragraph [0085] with the following amended paragraph:

[0085] The pooled product contained 1787 mg DNA, endotoxin level of 16 EU/mg, and 1.6% genomic DNA. The product was filtered again through [[0.2 m]] 0.2 μ nominal glass and [[0.2 m]] 0.2 μ absolute nylon filters described above. After filtering the product contained endotoxin level of 1 EU/mg and 0.18% genomic DNA, with 94% yield. The filtered product was diafiltered and subjected to a final [[0.2 m]] 0.2 μ sterilization filter as described in Example 1. The final product was 5.6 mg/ml, with 0.4 EU/mg and less than 0.2% genomic DNA.

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

Claims 1 – 22 (cancelled)

Claim 23 (New): A method for removing endotoxin from a plasmid DNA solution comprising:

a) contacting a solution comprising plasmid DNA with a trimethylamino ethyl (TMAE) anion exchange chromatography resin, the solution having a conductivity at which the plasmid DNA is bound to the resin;

b) washing the resin to elute endotoxin; and

c) eluting the plasmid DNA with a step or continuous gradient of increasing conductivity.

Claim 24 (New): The method of claim 23, wherein the TMAE anion exchange chromatography resin comprises a methacrylate based copolymer having a tentacle linked TMAE functional group.

Claim 25 (New): The method of claim 23, wherein the plasmid DNA solution is loaded on the resin in a solution having a conductivity of less than about 50 mS/cm.

Claim 26 (New): The method of claim 25, wherein the plasmid DNA is step eluted with a series of buffers of increasing conductivity in a range of from about 50 to about 90 mS/cm.

Claim 27 (New): The method of claim 23, further comprising a step of filtering the plasmid DNA solution through one or more filters selected from the group consisting of one or more glass fiber filters and nylon filters.

Claim 28 (New): The method of claim 27, where the plasmid DNA solution is filtered through a series of filters comprising at least one glass fiber filter and at least one nylon filter prior to contacting the plasmid DNA solution with the anion exchange chromatography resin.

Claim 29 (New): The method of claim 23, wherein the plasmid DNA solution is a clarified lysate obtained after alkaline lysis of bacterial cells comprising the plasmid DNA and removal of precipitated proteins, chromosomal DNA and cell debris.

Claim 30 (New): The method of claim 29, wherein the clarified lysate is further neutralized to a pH of about 7 to about 8.5.

Claim 31 (New): The method of claim 30, wherein the clarified lysate is further neutralized with a buffer that decreases an ionic strength of the lysate for direct loading onto the anion exchange resin.

Claim 32 (New): The method of claim 30, wherein the lysate is neutralized with a buffer that comprises Tris base.

Claim 33 (New): A method for removal of endotoxin from a plasmid DNA solution comprising:

a) filtering the plasmid DNA solution through a series of filters comprising at least one glass fiber filter and at least one nylon filter;

b) loading the filtered plasmid DNA solution onto a column comprising trimethylamino ethyl (TMAE) anion exchange resin, wherein the plasmid DNA solution is loaded onto the column in a loading buffer having a conductivity below which the plasmid DNA would elute from the resin;

c) washing the column with a buffer having a conductivity sufficient to elute endotoxin but not plasmid DNA from the resin; and

d) eluting the plasmid DNA with a step or continuous gradient of increasing conductivity, thereby producing a solution of anion exchange purified plasmid DNA.

Claim 34 (New): The method of claim 33, wherein the plasmid DNA solution comprises a clarified lysate obtained following alkaline lysis and precipitation using continuous flow static mixers.

Claim 35 (New): The method of claim 34, wherein the clarified lysate is neutralized to a pH of about 7 to about 8.5 prior to anion exchange chromatography.

Claim 36 (New): The method of claim 35, wherein the clarified lysate is neutralized with a buffer that deceases an ionic strength of the lysate for direct loading onto the anion exchange resin.

Claim 37 (New): A pharmaceutical scale method for purifying plasmid DNA comprising:

a) mixing a solution of bacterial cells comprising the plasmid DNA with an alkaline lysis solution by flowing through a first static mixer to obtain a lysate;

b) contacting the lysate with a potassium acetate precipitation solution by flowing through a second static mixer, thereby forming a precipitation mixture;

c) removing a precipitate from the precipitation mixture thereby forming a clarified lysate;

d) filtering the clarified lysate through a series of filters comprising at least one glass filter and one nylon filter thereby forming a filtered lysate;

e) loading the filtered lysate onto a trimethylamino ethyl (TMAE) anion ion exchange chromatography resin under conditions wherein the plasmid DNA is retained on the resin;

f) washing the resin with a buffer that removes weakly bound impurities from the resin and eluting the plasmid DNA with a step or continuous saline gradient, thereby producing a solution of anion exchange purified plasmid DNA.

Claim 38 (New): The method of claim 37, further comprising the step of RNase digestion.

Claim 39 (New): The method of claim 37, further comprising the step of adjusting the pH and conductivity of either the precipitation mixture or the clarified lysate to a pH in the range of about 7 to about 8.5 and a conductivity of less than about 50mS/cm prior to the filtering step wherein the filtered lysate can be directly loaded onto the anion ion exchange chromatography resin.

Claim 40 (New): The method of claim 37, wherein the trimethylamino ethyl (TMAE) anion ion exchange resin comprises a methacrylate based copolymer having a tentacle linked TMAE functional group.

Claim 41 (New): The method of claim 37, further comprising the step of purifying the plasmid DNA solution using ultrafiltration in the presence of a gel layer that is allowed to form before starting ultrafiltration.

Claim 42 (New): The method of claim 41, wherein the ultrafiltration unit is an open channel tangential flow ultrafiltration unit.

Claim 43 (New): A method for purifying plasmid DNA comprising:

a) lysing the bacterial cells by alkaline lysis and precipitation through continuous flow static mixers to provide a lysate;

b) clarifying the lysate and adjusting the pH and conductivity of the lysate to a pH of about 7.0 to about 8.5 and a conductivity of less than about 50mS/cm;

c) filtering the clarified and adjusted lysate through a filter series comprising a glass filter and a nylon filter to provide a filtered lysate;

d) purifying the plasmid DNA by anion exchange chromatography using a methacrylate based copolymer resin having a tentacle linked TMAE functional group; and

e) optionally, ultrafiltering and diafiltering the anion exchange purified plasmid DNA through a tangential flow open channel device in the presence of a gel-layer that is formed by an initial period of recirculation.

REMARKS/ARGUMENTS

For the convenience of the Examiner and the PTO, all reference to amendments to the specification and support for new claims relate to the paragraph numbering of the recent publication of the present application, US 2002/0198372 A1, attached here to.

I. Amendments to the Specification:

In the specification, the minor or obvious errors are corrected in paragraphs [0001], [0010], [0029], [0032], [0033 - 0034], [0040], [0041 - 0042], [0044], [0063 – 0065], [0069], [0083] and [0085].

In paragraph [0042], the following amendment is introduced to provide a chemical and structural definition of the claimed anion exchange resin, described by its trade name in the specification as TMAE Fractogel 650M: "TMAE Fractogel 650M is a tentacle ion exchanger having trimethylaminoethyl functional groups (TMAE) covalently attached to hydroxyl groups of a synthetic methacrylate based polymeric resin backbone." This description is abstracted from the manufacturer's brochure provided in the presently submitted Supplemental IDS and was known to those of skill in the art at the time of filing of the application. No new matter is introduced by this amendment.

II. Status of the Claims and Amendments:

Claims 1 – 22 have been cancelled and new claims 23 – 43 have been added.

The new claims do not introduce new matter. Support for the new claims can be found throughout the specification and drawings as originally found. For example, support for the new claims can be found, among other places, in the paragraphs indicated in tabular form below:

Claim and element	Support
Claim 23: trimethylamino ethyl (TMAE) anion exchange chromatography resin	Paragraphs [0042], [0071], and [0084]
eluting the plasmid DNA with a step or continuous gradient of increasing conductivity	Paragraphs [0041], [0043], and [0084]
Claims 24 and 40: the TMAE anion exchange chromatography resin comprises a methacrylate based copolymer having a tentacle linked TMAE functional group	Paragraph [0042] as amended in accordance with the manufacturer's brochure
Claim 25: plasmid DNA solution is loaded on the resin in a solution having a conductivity of less than about 50 mS/cm	Paragraph [0042]
Claims26 and 39: step eluted with a series of buffers of increasing conductivity in a range of from about 50 to about 90 mS/cm	Paragraph [0084]
Claims 27 and 28: filtering the plasmid DNA solution through one or more filters selected from the group consisting of one or more glass fiber filters and nylon filters	Paragraphs [0058], [0070], [0083]
Claim 29: the plasmid DNA solution is a clarified lysate obtained after alkaline lysis of bacterial cells	Examples 1 – 3, paragraphs [0064 – 86]
Claims 30, 35 and 39: the clarified lysate is further neutralized to a pH of about 7 to about 8.5	Paragraphs [0034], [0083]
Claims 31 and 36: clarified lysate is further neutralized with a buffer that deceases an ionic strength of the lysate for direct loading onto the anion exchange resin	Paragraphs [0034], [0043]
Claim 32: the lysate is neutralized with a buffer that comprises Tris base.	Paragraphs [0042], [0083]
Claim 33: A method for removal of endotoxin from a plasmid DNA solution comprising: a) filtering the plasmid DNA solution through a series of filters comprising at least one glass fiber filter and at least one nylon filter; b) loading the filtered plasmid DNA solution onto a column comprising trimethylamino ethyl (TMAE) anion exchange resin, wherein the plasmid DNA solution is loaded onto the column in a loading buffer having a conductivity below which the plasmid DNA would elute from the resin; c) washing the column with a buffer having a conductivity sufficient to elute endotoxin but not plasmid DNA from the resin; and d) eluting the plasmid DNA with a step or continuous gradient of increasing conductivity, thereby producing a solution of anion exchange purified plasmid DNA.	Paragraphs [0083 – 0084]
Claim 34: the plasmid DNA solution comprises a clarified lysate obtained following alkaline lysis and precipitation using continuous flow static mixers.	Paragraphs [0029 -34] and Example 2

Claim 37: A pharmaceutical scale method for purifying plasmid DNA comprising: a) mixing a solution of bacterial cells comprising the plasmid DNA with an alkaline lysis solution by flowing through a first static mixer to obtain a lysate; b) contacting the lysate with a potassium acetate precipitation solution by flowing through a second static mixer, thereby forming a precipitation mixture; c) removing a precipitate from the precipitation mixture thereby forming a clarified lysate; d) filtering the clarified lysate through a series of filters comprising at least one of a glass filter and a nylon filter thereby forming a filtered lysate; e) loading the filtered lysate onto a trimethylamino ethyl (TMAE) anion ion exchange chromatography resin under conditions wherein the plasmid DNA is retained on the resin; f) washing the resin with a buffer that removes weakly bound impurities from the resin and eluting the plasmid DNA from the resin with a step or continuous saline gradient, thereby producing a solution of anion exchange purified plasmid DNA.	Examples 1 -3, entire specification
Claim 38: RNase digestion	Paragraphs [0029], [0064]
Claim 39: adjusting the pH and conductivity of either the precipitation mixture or the clarified lysate to a pH in the range of about 7 to about 8.5 and a conductivity of less than about 50mS/cm prior to the filtering step and wherein the filtered lysate can be directly loaded onto the anion ion exchange chromatography resin.	Paragraphs [0034], [0042-43], [0083]
Claim 41: using ultrafiltration in the presence of a gel layer	Paragraphs [0045 -56] and Example 1
Claim 42: the ultrafiltration unit is an open channel tangential flow ultrafiltration unit.	Paragraphs [0017], [0021], [0049], [0066]
Claim 44: A method for purifying plasmid DNA comprising: a) lysing the bacterial cells by alkaline lysis and precipitation through continuous flow static mixers to provide a lysate; b) clarifying the lysate and adjusting the pH and conductivity of the lysate to a pH of about 7.0 to about 8.5 and a conductivity of less than about 50mS/cm; c) filtering the clarified and adjusted lysate through a filter series comprising a glass filter and a nylon filter to provide a filtered lysate; d) purifying the plasmid DNA by anion exchange chromatography using a methacrylate based copolymer resin having a tentacle linked TMAE functional group; and e) optionally, ultrafiltering and diafiltering the anion exchange purified plasmid DNA through a tangential flow open channel device in the presence of a gel-layer that is formed by an initial period of recirculation.	Examples 1 – 3, and entire specification

III. Double Patenting

All prior pending claims have been cancelled and new claims added. Applicant respectfully submits that all new claims are patentably distinct from those of US 6,011,148 and requests reconsideration of Examiner's prior double patenting rejection. Applicant would in any event respectfully request a deferral of any requirement for a terminal disclaimer until such time as claims are allowed.

IV. Rejections under 35 U.S.C. § 103

While not conceding the Examiner's determination of obviousness of the prior pending claims, in order to advance the prosecution of the present case all prior rejected claims have been cancelled. New claims have been added to clarify the claimed invention. The present claims are clearly distinguished over the prior art. All of the new independent claims directed to methods of plasmid DNA purification utilize a step of anion exchange chromatography using a resin having a trimethylaminoethyl (TMAE) functional group. This functional group forms a strong anion exchanger. Because DNA is an anion, strong anion exchangers were avoided in some plasmid purification protocols because the plasmid DNA could be difficult to recover from the column. *See, e.g.* AU199721661B2, Australian English language counterpart of WO97/35002, entitled "Purification of Pharmaceutical-Grade Plasmid DNA", included in the present Supplemental IDS.

Applicant surprising found that ion exchange chromatography of plasmid DNA solutions using the TMAE Fractogel anion exchange resin resulted in significant purification, particularly through the removal of endotoxins, and that the capacity of the resin made it suitable for large scale manufacturing. Applicant is not aware of any prior art that taught or suggested the present claimed method of plasmid purification using TMAE anion exchange chromatography.

To the extent the independent claims are patentable, the dependent claims are have been similarly placed in condition for allowance.

Conclusion

For the reasons stated herein, the Applicant respectfully submits that independent claims 23, 33, 37 and 43 are allowable and that the dependent claims are, in turn, also allowable. Applicant respectfully requests allowance of the claims at an early date. The Commissioner is

authorized to charge any additional fees incurred in this application or credit any overpayment to Deposit Account No. 50-1922. Should the Examiner have any questions, please do not hesitate to call Applicant's attorney at 832-446-2421.

Respectfully submitted,

By _____
Marilyn M. Huston
Reg. No. 37,851
Wong, Cabello, Lutsch, Rutherford & Brucculeri, L.L.P.
20333 SH 249, Suite 600
Houston, TX 77070
(832) 446-2421

CERTIFICATE OF MAILING UNDER 1.11(a)

I hereby certify that this document is being deposited with the U.S. Postal Service Express Mail Service in an envelope addressed to Mail Stop RCE, Commissioner for Patents, P. O. Box 1450, Alexandria, VA 22313-1450 on August 29, 2003.

_____
8-29-03